ARTHROPOD SYSTEMATICS & PHYLOGENY

SENCKENBERG

The African-Iberian connection in Odonata: mtDNA and ncDNA based phylogeography of *Aeshna cyanea* (Müller, 1764) (Odonata: Aeshnidae) in Western Palaearctic

THOMAS J. SIMONSEN^{*,1}, KENT OLSEN¹ & MARIE DJERNÆS¹

¹ Natural History Museum Aarhus, Wilhelm Meyers Allé 10, DK-8000 Aarhus, Denmark; Thomas Simonsen [t.simonsen@nathist.dk]; Kent Olsen [kent@molslab.dk]; Marie Djernæs [marie_djernaes@hotmail.com] — * Corresponding author

Accepted on September 9, 2020.

Published online at www.senckenberg.de/arthropod-systematics on September 24, 2020.

Editors in charge: Christiane Weirauch & Anna Hundsdörfer

Abstract. We explore the phylogeography and inter-population relationships of the Southern Hawker dragonfly, Aeshna cyanea (Müller) in the Western Palaearctic region based on 603 bp Cytochrome Oxidase Subunit 1 (COI) mtDNA and 732 bp Internal Transcribed Spacer region (Internal Transcribed Spacer 1, 5.8S ribosomal RNA gene and Internal Transcribed Spacer 2, ITS region) ncDNA with an increased sampling from Europe compared to a previous study. Both DNA fragments recover a remarkable and compatible pattern: the recently described Aeshna vercanica Schneider et al. is the sister group of A. cyanea, which in turn comprises three distinct populations. These populations are: a population in the Caucasus region; a North African population; and a European population. When analysed alone, the ITS fragment recovered A. vercanica and the Caucasus A. cyanea population as separate units, but the North African and European A. cyanea populations were recovered as intermixed. F_{ST} population genetic analyses of COI data revealed high degrees of isolation between all populations as all inter-population values were between 0.818 (North Africa – Europe) and 0.944 (Europe – A. vercanica). Average pairwise distance in COI (uncorrected p) between populations followed this pattern and was lowest between Europe and North Africa and highest between North Africa and A. vercanica, and between Europe and A. vercanica. Within population pairwise distance values were approximately an order of magnitude lower. Pairwise distance values between populations for the ITS region were much lower than for COI, but followed the same pattern. Our results therefore support the full species status for A. vercanica, and clearly indicate that the current Western European A. cyanea population originated from a North African galcial refugium and dispersed to Europe (the Iberian Peninsula) prior to the Holsteinian interglacial period. While the North African and European populations likely remained in contact initially, the European population was probably isolated in the Iberian Peninsula during the Holsteinian interglacial period, and subsequently spread throughout Europe in late Pleistocene - early Holocene.

Key words. Odonata, Aeshna, phylogeography, refugia, glacial cycles.

1. Introduction

Current European biogeography is strongly affected by the Quaternary glacial cycles, with dramatic climate fluctuations over the past two million years having left complex patterns of range expansions and contractions, as well as speciation and extinction events (e.g. HEWITT 1999; SCHMITT 2007). The past couple of decades have seen a surge in species and population level biogeographical studies of the European flora and fauna (e.g. HEWITT 1999, 2004; SCHMITT 2007; NORMAND et al. 2011; SIMONSEN & HUEMER 2014; HUSEMANN et al. 2014). The emerging picture is a mixture of patterns where some studies reveal patterns that confirm the presence of major Mediterranean glacial refugia in the Iberian, Italian and Balkan peninsulas (see DE LATTIN 1964, 1967; HEWITT 2004 for general patterns) in plants (VON CRÄUTLEIN et al., 2019), vertebrates (URSENBACHER et al. 2006; JOGER et al. 2007; NEBEL et al. 2015; MARZAHN et al. 2016) and hexapods (VILA et al. 2005; LOUY et al. 2013; HINOJOSA et al. 2017) – some of these studies have demonstrated several Mediterranean refugia in the same species. However, a number of studies show markedly different patterns, which include patterns indicating northern glacial refugia (e.g. SCHMITT et al. 2007; GRATTON et al. 2008; HAMMAOUTI et al. 2009; SLOVÁK et al. 2012; BESOLD & SCHMITT 2014;



SIMONSEN & HUEMER 2014; DANECK et al. 2015; PAUĆU-LOVA et al. 2016; WINDMAISSER et al. 2016; QUINZIN et al. 2017). Others indicate the presence of refugia in eastern or southeast Western Palaearctic (SADEGHI et al. 2010; SZTENCEL-JABŁONKA et al. 2015; KAJTOCH et al. 2016; KAMP et al. 2019), while some studies indicate a rapid post glacial expansion across the entire Palaearctic (BERNARD et al. 2011) or even Holarctic (NEBEL et al. 2015; KOHLI et al. 2018) regions from eastern Siberian refugia. Until recently, the role North African refugia may have played in Western Palaearctic Quaternary biogeography has been somewhat overlooked, not least the routes through which Europe has been colonised from such North African refugia (HABEL et al. 2001; DAPPORTO et al. 2001; HUSEMANN et al. 2014; EL MOKHEFI et al. 2016; LEFEBVRE et al. 2016). Understanding these patterns and their timing remains crucial not only for understanding current European biogeography, but also for predicting the effects of current and future climatic changes on biodiversity.

Insects are often a focus group for zoological phylogeography because of their significant biological diversity, fast generation time and large population sizes - which mean they often respond quickly to changes - and general ease of access. Indeed, many studies on European phylogeography and speciation are based on insect groups (several of the studies mentioned above as well as others), mostly Lepidoptera (butterflies and moths), and Orthoptera (grasshoppers and crickets). Another insect order of considerable interest for phylogeography and diversification studies are Odonata (dragonflies and damselflies): they are ecologically very diverse, but the individual species are often strongly tied to specific ecological conditions, and all have highly complex life cycles with aquatic juvenile stages and terrestrial adults. This means that they link the terrestrial and aquatic environments, and are doubly sensitive to environmental and climatic changes - they react to changes in both environments and not just one of them (e.g. BYBEE et al. 2016). Several studies have indeed focused on phylogeography and/or species delimitation of Western Palaearctic Odonata. ARTISS (2004) found strong genetic separation between Nearctic and Palaearctic Libellula quadrimaculata Linneaus, 1758, but only differentiation between Japan and the remaining Palaearctic – not within the Palaearctic region in general. KOHLI et al. (2018) found divergence in the circumpolar arctic Somatochlora salbergi Trybom, 1889 between Japan (Hokkaido) and the rest of the populations, but otherwise no variation. Similarly, BERNARD et al. (2011) found very limited divergence across the Palaearctic region in Nehalennia speciosa (Charpentier, 1840). In contrast to this, HINOJOSA et al. (2017) found a clear pattern in Sympetrum vulgatum (Linneaus, 1758), which is divided into three geographical groups: Iberian Peninsula; Europe; and Caucasus. The closely related damselflies Coenagrion puella (Linneaus, 1758) and C. pulchellum (Vander Linden, 1825) share intermixed COI haplotypes in Europe (FREELAND & CONRAD 2002), and cannot be separated based on mtDNA. However,

they are well separated based on nuclear microsatellite markers (LowE et al. 2008), demonstrating clearly the risks of relying on mitochondrial genes only.

Recently, SCHNEIDER et al. (2015) studied the Aeshna cyanea (Müller, 1764) complex and described a new species, A. vercanica Schneider et al., 2015, based on distinctive specimens from the Hyrcanian Forest region in northwest Iran and southeast Azerbaijan (Fig. 1). When they analysed molecular differences in both mitochondrial and nuclear genetic markers between A. vercanica and A. cvanea, they discovered a distinctive pattern in the latter with clear distinction between populations in the Caucasus, North Africa, and Europe. As their focus was on the description of the new species, A. vercanica, they did not explore the pattern in A. cyanea further, but noted that the African population may be a glacial relict (SCHNEIDER et al. 2015). Aeshna cyanea is a distinctive, versatile and mobile species, which occurs widespread in Western Europe from Gibraltar and Sicily to northern England, southern Scandinavia and southern Finland. It is absent from Ireland, and rare and localised in Scotland. While it is apparently absent from south eastern Italy and southern Balkan, it probably occurs throughout the rest of Eastern Europe to the Ural and Caucasus mountains, although the exact distribution is poorly known. It is absent from most of Anatolia, the Middle East, and north eastern Africa, but there are rare and localised populations in north western Africa (Fig. 1; Askew 2004; Sch-NEIDER et al. 2015; KALKMAN & KITANOVA 2015). Here we augment SCHNEIDER et al.'s (2015) data set with specimens from northern and Eastern Europe to explore the phylogenetic and phylogenetic patterns, population genetics, and molecular divergence times in the A. cyanea complex in relation to Quaternary glacial cycles in the Western Palaearctic.

2. Material and Methods

2.1. Material

We sampled sixteen specimens of Aeshna cyanea from Europe. These samples were augmented with Genbank sequences from a further six European specimens, three North African specimens, nine specimens from the Caucasus region and three A. vercanica specimens from Iran/ Azerbaijan. All Genbank sequences were included in SCHNEIDER et al.'s (2015) initial study of A. cvanea/vercanica. A single specimen of each of the species Aehsna juncea (Linneaus, 1758) and Aeshna mixta Latreille, 1805 were used as outgroups. The full data set thus comprise 39 specimens. All specimen data is given in Table 1. All voucher specimens sequenced for this study are deposited at the Natural History Museum Aarhus. The distribution and sample localities map in Fig. 1 was constructed by exporting a blank map from DIVA-GIS 7.5.0.0 using the global country boundaries dataset provided on the



Fig. 1. Geographic range and sampling sites for *Aeshna cyanea* (inserted photo) and *A. vercanica*. Distribution of *A. cyanea* is shown in grey, while distribution of *A. vercanica* is shown in black. Open circles indicate sampling sites in Schneider et al. (2015), while filled circles indicate our sampling sites. Small circles indicate a single specimen, while large circles indicate several specimens. Modified from Schneider et al. (2015).

program's website (HIJMANS et al. 2012). Species distributions (based on SCHNEIDER et al. 2015; KALKMAN & KI-TANOVA 2015), and sample localities (based on SCHNEIDER et al. 2015 and our sample data) were added manually in Adobe Photoshop CS2.

2.2. Methods

2.2.1. Laboratory procedures

Following SCHNEIDER et al. (2015) we targeted the barcode region of the mitochondrial gene COI (HEBERT et al. 2003), and the full Internal Transcribed Spacer region (ITS region) in the nuclear genome. This allow us to utilise the sequences published by SCHNEIDER et al. (2015) and build directly on their work. COI has been widely used in species delineation and phylogeograpic studies in insects (e.g. GRATTON et al. 2008; SIMONSEN et al. 2008; HAMMAOUTI et al. 2009; HUEMER 2011; MUTANEN et al. 2012; SIMONSEN & HUEMER 2014), although the use of a single, mitochondrial gene for such studies has been criticised (e.g. Dupois et al. 2012; KONDANDARAMAIAH et al. 2013; SIMONSEN et al. 2019). The nuclear ITS region, which comprises the genes ITS1, 5.8S, and ITS2 has been used less frequently, but shows considerable inter- and intra-specific variation in insects (e.g. WEEKERS et al. 2001; JÖDICKE et al. 2003; CHOBANOV et al. 2016). As haplotype network analyses are sensitive to missing data, we trimmed the data sets to make sure that there

was no missing data. The aligned combined data set is available as Supplementary Material S1.

DNA was extracted at Aarhus University (AU), Denmark using either E.Z.N.A. Tissue DNA Kit (Omega BIO-TEK) or DNeasy Blood & Tissue Kit (Qiagen). The E.Z.N.A. Tissue DNA Kit protocol was followed with some modifications: Samples were incubated at 42° C for 18-23 hours during lysis, steps 5 and 6 in the protocol were skipped, and samples were incubated with Elution Buffer for 5-10 min at 70° C and eluted once in 200 µl. The DNeasy Blood & Tissue Kit protocol was followed with some modifications: Samples were incubated at 42° C for 20 hours during lysis, elution buffer AE was heated to 60° C prior to elution, samples were incubated with buffer AE for 10 min at 60° C and eluted once in 100 µl. The lower lysis temperature compared to the 55–56° C in the manufacturers' protocols, follows KROSCH & CRANSTON (2012), who demonstrated that a lower lysis temperature is suitable for longer lysis times.

We used the following PCR protocol for COI: 95° C, 2 min; then 35–45 cycles of 95° C, 30 s; 45° C, 30 s; 72° C, 1 min and a final extension of 72° C for 5 min using the primers OdoF2 (with universal tail): **TGTAAAACGACGGCCAGT**TTTCTACAAAYCAY AARGATATTGG (tail in boldface italics); and OdoR3 (with universal tail): **CAGGAAACAGCTATGAC**TAAA CYTCTGGRTGRCCAAARAATCA (tail in boldface italics). We used the same PCR protocol for ITS using the primers VRAIN2F (with universal tail): **TGTAAAACGA**

Genus	Species	Geogroup	Country	Region	Voucher	Reference	Genbank# COI	Genbank # ITS
Aeshna	juncea	,	Denmark	West Jutland	ENT-DNA-249	New	MN847868	MN993616
Aeshna	mixta		Denmark	East Jutland	ENT-DNA-172	New	MN939165	MN993632
Aeshna	vercanica		Iran	I	ve1	Schneider et al. 2015	KU 180302	KU180365
Aeshna	vercanica		Iran	I	ve2	Schneider et al. 2015	KU 180303	KU180368
Aeshna	vercanica		Azerbaijan	Lankaran	ve3	Schneider et al. 2015	KU180322	KU180361
Aeshna	cyanea	Caucasus	Armenia		c18	Schneider et al. 2015	KU 180315	KU180369
Aeshna	cyanea	Caucasus	Armenia	1	c2	Schneider et al. 2015	KU180316	KU180370
Aeshna	cyanea	Caucasus	Armenia	1	c5	Schneider et al. 2015	KU180319	KU180371
Aeshna	cyanea	Caucasus	Armenia	1	c12	Schneider et al. 2015	KU180321	KU180372
Aeshna	cyanea	Caucasus	Georgia	1	c17	Schneider et al. 2015	KU180314	KU180375
Aeshna	cyanea	Caucasus	Russia	Mostovsky, Caucasus	c15	Schneider et al. 2015	KU180312	KU180380
Aeshna	cyanea	Caucasus	Russia	Mostovsky, Caucasus	c16	Schneider et al. 2015	KU180313	KU180381
Aeshna	cyanea	Caucasus	Russia	Mostovsky, Caucasus	c1	Schneider et al. 2015	KU180317	KU180382
Aeshna	cyanea	Caucasus	Russia	Mostovsky, Caucasus	c4	Schneider et al. 2015	KU180318	KU180383
Aeshna	cyanea	North Africa	Algeria		c14	Schneider et al. 2015	KU180311	KU180374
Aeshna	суапеа	North Africa	Tunesia		c13	Schneider et al. 2015	KU180310	KU 180385
Aeshna	суапеа	North Africa	Tunesia	1	c6	Schneider et al. 2015	KU180320	KU 180386
Aeshna	суапеа	Europe	Belgium	1	c7	Schneider et al. 2015	KU180304	KU180373
Aeshna	суапеа	Europe	Germany	Baden-Württemberg	c10	Schneider et al. 2015	KU180307	KU 180376
Aeshna	суапеа	Europe	Italy		c8	Schneider et al. 2015	KU180305	KU180377
Aeshna	суапеа	Europe	Poland	West Pomerania	c9	Schneider et al. 2015	KU180306	KU 180378
Aeshna	суапеа	Europe	Poland	West Pomerania	c11	Schneider et al. 2015	KU180308	KU 180379
Aeshna	суапеа	Europe	Spain		c3	Schneider et al. 2015	KU180309	KU 180384
Aeshna	суапеа	Europe	Poland	Biala Podlaska	ENT-DNA-1131	New	MN939093	MN963665
Aeshna	суапеа	Europe	Sweden	Öland	ENT-DNA-989	New	MN939088	MN963656
Aeshna	суапеа	Europe	Denmark	Bornholm	ENT-DNA-135	New	MN939079	MN963667
Aeshna	суапеа	Europe	Denmark	Bornholm	ENT-DNA-136	New	MN939080	MN963658
Aeshna	суапеа	Europe	Denmark	Bornholm	ENT-DNA-344	New	MN939085	MN963666
Aeshna	суапеа	Europe	Denmark	Bornholm	ENT-DNA-345	New	MN939086	MN963668
Aeshna	суапеа	Europe	Denmark	Bornholm	ENT-DNA-346	New	MN939087	MN963662
Aeshna	суапеа	Europe	Denmark	Northeast Zealand	ENT-DNA-347	New	MN939090	MN963670
Aeshna	суапеа	Europe	Denmark	Northeast Zealand	ENT-DNA-348	New	MN939091	MN963663
Aeshna	суапеа	Europe	Denmark	Northeast Zealand	ENT-DNA-349	New	MN939092	MN963664
Aeshna	cyanea	Europe	Denmark	Møn	ENT-DNA-342	New	MN939083	MN963669
Aeshna	cyanea	Europe	Denmark	Fyn	ENT-DNA-332	New	MN939089	MN963660
Aeshna	cyanea	Europe	Denmark	East Jutland	ENT-DNA-112	New	MN939078	MN963657
Aeshna	cyanea	Europe	Denmark	East Jutland	ENT-DNA-343	New	MN939084	MN963661
Aeshna	cyanea	Europe	Denmark	South Jutland	ENT-DNA-176	New	MN939082	MN963659
Aeshna	cyanea	Europe	Denmark	West jutland	ENT-DNA-175	New	MN939081	MN963655

in boldface italics); and VRAIN2R (with universal tail): *CAGGAAACAGCTATGAC*TTTCACTCGCCGTTA CTAAGGGAATC (tail in boldface italics). The COI primers were developed for this study, while the ITS region primers were from FÉLIX et al. (2001). All samples were sequenced at Macrogen Europe using the Sanger Method. Contigs and consensus sequences were obtained using DNA Baser Sequence Assembler v5.8.0 (HERACLE BIOSOFT 2018). We checked the identity of all sequences using BLAST on GenBank and/or BOLD (Barcode Of Life Data base) Identification System.

2.2.2. Phylogenetic analysis and molecular dating

We analysed the combined COI + ITS data set in Mr-Bayes v3.2.5 (RONQUIST et al. 2012). The data set was divided into two partitions (COI and ITS region), for each partition we allowed the program to assess the best model for evolution (nst=mixed) with a gamma distribution. The analysis was run for 100 million generations with sampling every 10,000 generations, and the first 50% were discarded as burnin. We allowed Mr Bayes to access the best model rather than using a dedicated software package for this purpose following the recommendation in the Mr Bayes v3.2.5 manual (RONQUIST et al. 2012). To avoid potential over-parameterisation, we did not partition the COI dataset into codon partitions.

We ran molecular dating analyses in BEAST v2.6 in conjunction with BEAUti v2.6 (BOUCKAERT et al. 2019). As in MrBayes the data was partitioned into COI and the ITS region, while the substitution model for both partitions was set to GTR with a gamma distribution based on the model assessed by MrBayes, and the tree prior set to the Yule Model; all other priors were left as default. We first ran an exploratory analysis in BEAST for 100 million generations with sampling every 10,000 generation to compare the result to the result obtained from the analysis in MrBayes. We then fixed the topology in BEAST to correct the major difference between the analyses (i.e. including the Spanish specimen in A. cyanea Europe see Results), and set the clock to Relaxed Exponential with the clock rate left to 1.0, and the age of their last common ancestor was set to 1.5 million years (based on an uncorrected p COI divergence rates of 0.036 - see Results) with a sigma value of 1.0. The new analysis was run for 10 million generations. To test the stability of the result we reran the latter analysis twice with the age of the last common ancestor of A. vercanica + A. cyanea set to 1.0 million years and 2.0 million years, respectively. In the two latter analyses the sigma values were set to 0.5 and 1.5, respectively. In all BEAST analyses we constructed a consensus tree in TreeAnnotator 2.6.0 (part of the BEAST package) discarding the first 50% of the trees as burnin. The log files from MrBayes and BEAST were examined in Tracer 1.6.0 (part of the BEAST package), and trees were analysed in FigTree 1.4.2 (part of the BEAST package).

2.2.3. Phylogeographic analyses, genetic diversity and population genetics

Prior to running the phylogeographic and population genetic analyses, we removed outgroups, otherwise the data sets were identical to the data sets used for phylogenetic analyses. We constructed median-joining haplotype networks (BANDELT et al. 1999) for the individual COI and ITS data sets, and for the combined data set in Network v5.0.1.1 (available at: fluxus-engineering.com).

We separated the samples into populations to explore genetic diversity and population genetic patterns. We treated A. vercanica (3 specimens) as a single population, whereas A. cyanea was split into three population geo-groups based on geography: A. cvanea Caucasus (9 specimens), A. cyanea North Africa (3), and A. cyanea Europe (22) (Table 1). We calculated the average uncorrected p distance for each gene individually between populations and within populations in MEGA 7 (KUMAR et al. 2016). The uncorrected p distance has been used in a wide range of studies, and employing it here allows for direct comparison to earlier results. The within and between population values for COI and the ITS region are shown in Tables 2 and 3, respectively. To assess how isolated the populations are we calculated the F_{ST} value between populations based on the COI data set in Arlequin 3.5.2.2 (Excoffier et al. 2005) with 10,000 permutations to test for statistical significance. F_{ST} values are shown in Table 4.

3. Results

3.1. Phylogenetics and phylogeography

The 39 taxa data set for the phylogenetic analyses was comprised by 603 bp COI, and 733 bp ITS region including gaps. The 37 taxa data set for the haplotype network analyses was comprised by 603 bp COI and 702 bp ITS. The difference in the ITS data set length corresponds to gaps in the alignment that includes outgroup taxa, whereas without outgroups, the alignment had no gaps. 16 different haplotypes were identified in the ingroup in the COI data set, and eleven haplotypes were identified in the ingroup in the ITS region data set.

The analysis in MrBayes (Supplementary Figure S2) recovered the 'A. cyanea-group' (A. vercanica + A. cyanea) as monophyletic with strong support (pp=1), just as A. vercanica and A. cyanea are recovered as reciprocally monophyletic with strong support (pp=1 and 0.996, respectively). Aeshna cyanea Caucasus is not recovered as monophyletic, but forms as paraphyletic grade at the base of A. cyanea. Aeshna cyanea Europe and A. cyanea North Africa are recovered as reciprocally monophyletic sister groups in a well-supported western clade (pp=0.9998), but support for a monophyletic A. cyanea Europe group is very low (pp=0.52), while support for a monophyletic A. cyanea North Africa group is strong (pp=1). The



Fig. 2. Median-joining haplotype networks of *Aeshna cyanea* and *A. vercanica*. Numbers next to a branch indicate number of mutations. A: COI + ITS region; B: COI; C: ITS region.

unconstrained analysis in BEAST (Supplementary Figure S3) returned an overall similar result with two important differences: 1) *A. cyanea* Caucasus is recovered as monophyletic with strong support (pp=0.9892) and comprise the sister group of the remaining *A. cyanea*; 2) The single Spanish specimen is removed from *A. cyanea* Europe and is placed as sister to *A. cyanea* North Africa. However, the support for this placement is poor (pp=0.6589). The support for the monophyly of the remaining European specimens remains poor (pp=0.6709).

The median-joining haplotype networks display the same overall pattern (Fig. 2). In the network based on the combined data set (Fig. 2A) *A. vercanica* is connected to the remaining network by a very long branch that connects to a median vector. *Aeshna cyanea* Caucasus connects to the same median vector by a short branch,

which also connects via a long branch to an overall group comprising the North African and European specimens. Aeshna cyanea North Africa connects directly by a long branch to the complex A. cyanea Europe. The network based on the COI data set (Fig. 2B) shows the same general relationships, with the only difference being that A. cyanea North Africa via a medium length branch are connected to a median vector they share with the Spanish and one Polish specimen. This group is connected to the remaining European specimens. The network based on the ITS region data set (Fig. 2C) is somewhat different, but overall compatible to the other analyses; while A. vercanica and A. cyanea Caucasus are still separated from the remaining specimens, the European and North African specimens are not separated as the North African specimens and a single Belgian specimen share a unique

COI, average	Europe	North Africa	Caucasus	A. vercanica
Europe	0.002			
North Africa	0.013	0.004		
Caucasus	0.016	0.021	0.001	
A. vercanica	0.039	0.044	0.028	0.004

Table 2. Average uncorrected p values for COI within and between geo-groups of A. cyanea, and A. vercanica.

Table 3. Average uncorrected p values for the ITS region within and between geo-groups of A. cyanea, and A. vercanica.

ITS, average	Europe	North Africa	Caucasus	A. vercanica
Europe	0.002			
North Africa	0.002	0.000		
Caucasus	0.005	0.003	0.000	
A. vercanica	0.008	0.006	0.006	0.000

Table 4. Pairwise F_{sT} values between geo-groups of A. cyanea, and A. vercanica. Numbers in boldface are statistically significant.

Fst, pairwise	Europe	North Africa	Caucasus
North Africa	0.81849		
Caucasus	0.89558	0.91336	
A. vercanica	0.94409	0.90000	0.93460

haplotype. The combined *A. cyanea* Europe and *A. cyanea* North Africa group remain separated from *A. vercanica* and the *A. cyanea* Caucasus group.

3.2. Genetic diversity

The uncorrected p distance for COI (Table 2) within geogroups is very low and ranged from 0.001 to 0.004. The same distance between populations was approximated an order of magnitude higher ranging from 0.013 to 0.044, but there were considerable differences between the different populations. A. cyanea Europe and A. cyanea North Africa were most similar with a value of 0.013, while the distance between A. cyanea Europe and A. cyanea Caucasus, and A. cyanea North Africa and A. cyanea Caucasus were 0.016, and 0.021, respectively. Not surprisingly, A. vercanica and the various A. cyanea showed the greatest differences. However, the distance values varied from 0.028 between A. vercanica and A. cyanea Caucasus, to 0.039 and 0.044 between A. vercanica and A. cyanea North Africa, and A. vercanica and A. cyanea Europe, respectively. The average uncorrected p distance between A. vercanica and all A. cyanea was 0.036. The uncorrected p distance for the ITS region (Table 3) within populations was 0 for all populations except A. cyanea Europe, where it was 0.002. The uncorrected p distance between populations was low between all populations, but lower between A. cyanea populations (0.002–0.005) than between A. vercanica and the different A. cyanea populations (0.006-0.008). The pairwise F_{ST} values (Table 4) are high (0.81849 - 0.94409) between all populations. F_{ST} values range from 0.0 to 1.0 and indicates how isolated two populations are from each other with 0.0 indicating no isolation with free interbreeding, and 1.0

indicating full isolation and no interbreeding (e.g. MEIR-MANS & HEDRICK 2011). Our results thus indicate that all the populations display a high degree of isolation, with *A. cyanea* Europe and *A. cyanea* North Africa with an F_{ST} value of 0.82 being least isolated, while the other population pairs, including *A. vercanica* and the different *A. cyanea* populations, show a higher and similar degree of isolation ($F_{ST} \approx 0.90-0.94$).

3.3. Divergence times

As outlined above we used an enforced topology in the divergence time analysis in BEAST (Fig. 3). Aeshna cyanea Caucasus is non-monophyletic in the MrBayes analysis, but monophyletic with strong support in the BEAST analysis. The group also appear united in all the haplotype analyses, and the average uncorrected p value between the specimens in the group is the lowest of all the groups. We therefore allowed it to remain monophyletic in the divergence time analysis without any constrains. However, we enforced monophyly of all European specimens including the single specimen from Spain. The Spanish specimen grouped with the North African specimens in the unconstrained BEAST analysis, but it was nested within the European specimens in the MrBayes analysis (albeit with weak support in both analyses). It also appears closer associated with the European specimens in all three haplotype network analyses. As there are no fossil records that can be assigned to the A. cyanea group, and no phylogeny of Aeshna with divergence times has been published, we rely on COI divergence to calibrate the dating analysis in BEAST. Following BROWER'S (1994) estimate of an average COI evolution rate of 2% pr. million year, we set the split between



Fig. 3. Timed phylogram from the 10 million generation constrained BEAST analysis. Timing of the major splits discussed in the text are indicated by arrows. Numbers above a branch are posterior probabilities from Mr Bayes, while numbers below a branch are posterior probabilities from BEAST.

A. vercanica and A. cyanea (uncorrected p=0.036) to 1.5 million years +/- 1.0 million years, with settings otherwise as explained above. The resulting tree with the dating values for the major splits is shown in Fig. 3. The split between A. cyanea and A. vercanica is estimated to be ca 0.82 mya (0.15–1.90 mya); the split between A. cyanea Caucasus, and A. cyanea North Africa + A. cyanea Europe is estimated to be ca 0.61 mya (0.106–1.45 mya), while the split between A. cyanea North Africa and A. cyanea Europe is estimated to be ca 0.41 mya (0.07–0.98 mya). The divergence times were only slightly higher in the analysis where the split between A. vercanica and A. cyanea was set to 2.0 million years, and slightly lower when it was set to 1.0 million years (results not shown).

4. Discussion

Our results confirm the conclusion by SCHNEIDER et al. (2015) that *A. vercanica* and *A. cyanea* form two distinct and well-separated clades. While SCHNEIDER et al. (2015) were not able to resolve the relationship between *A. vercanica*, *A. cyanea* Caucasus, and *A. cyanea* North Africa + *A. cyanea* Europe, our results clearly place *A. vercanica* as the sister taxon of *A. cyanea*. The very high pairwise F_{ST} values (Table 4) between *A. vercanica* and the three *A. cyanea* geo-groups ($F_{ST} \ge 0.9$) indicating virtually no interbreeding between *A. vercanica* and *A. cyanea* also support the full species status of the former. Interestingly the lowest pairwise F_{ST} value (0.9) is

between A. vercanica and A. cyanea North Africa, and this is also the only pairwise F_{st} value that is not statistically significant. Given the considerable geographical and phylogenetic distance between the two groups, we consider this most likely an analytical abnormality, possibly due to the low sample size of the two populations. We also confirm SCHNEIDER et al.'s (2015) initial observations that A. cyanea is divided into three distinct and geographically separated sub-clades: one in Caucasus; one in northwest Africa; and one in Europe. Although our Bayesian analysis in MrBayes (Supplementary Figure S2) fail to recover A. cyanea Caucasus as monophyletic (a result similar to SCHNEIDER et al. (2015)), the Bayesian analysis in BEAST (Supplementary Figure S3), and all haplotype network analyses (Fig. 2A-C) recover the group as monophyletic, and distinct from the other A. cyanea geo-groups. SCHNEIDER et al. (2015) considered the Caucasus group to be a loosely related group, but our results – especially the very low internal uncorrected pdistance (Tables 2-3), and the high F_{ST} values (Table 4) compared to other groups - indicate that the group is both well-defined and isolated. SCHNEIDER et al. (2015) considered A. cyanea North Africa and A. cyanea Europe to comprise a single subclade related to the western Mediterranean refugium. Although our results confirm that the two groups are closely related and form a distinct clade, there are also clear indications that they form two separate groups. Our Bayesian analyses have difficulties placing the Spanish specimen clearly in one of the groups with the unconstrained BEAST analysis placing it with the North African group (Supplementary Figure S3), albeit with very low support (pp ≈ 0.65), and MrBayes placing it with the European group (Supplementary Figure S2) with very low support ($pp \approx 0.52$). However, the haplotype networks do place it with the other European specimens (Fig. 2A–C), and while the pairwise F_{ST} value between A. cyanea North Africa and A. cyanea Europe (including Spain) is the lowest in the analysis, it is still very high (0.81849) and indicate a high degree of isolation between the groups (Table 4).

It is interesting to compare out results to HINOJOSA et al.'s (2017) study of another dragonfly, Sympetrum vulgatum. This species is widespread in the Palearctic region and divided into three subspecies: S. vulgatum decoloratum in Caucasus and Anatolia; S. vulgatum ibericum in the Iberian Peninsula; and S. vulgatum vulgatum in the rest of Europe (HINOJOSA et al. 2017). Based on COI and the ITS region they found that the three subspecies are separated, but that the genetic divergence in COI is low (0.1-0.4%), while there was no difference in the ITS region. Interestingly, they found that S. vulgatum vulgatum from Europe and S. vulgatum decoloratum from Caucasus and Anatolia were more similar to each other (COI divergence = 0.1%) than either were to S. vulgatum ibericum from Spain (COI divergence = 0.3-0.4%). Their results are in other words more or less the opposite of ours. It is also worth noting that the three well-defined subspecies in S. vulgatum display an intersubspecific variation in COI that is directly comparable to the *intra*-population variation we find in the *A. cyanea* group.

The deep split between populations in the Caucasus, and populations in Europe in general was also found by KAMP et al. (2018) in a detailed study of the Middle-Spotted Woodpecker based on several mitochondrial and nuclear markers. Several studies over the past few years have found evidence of a Pleistocene glacial refugium in Northern Africa (reviewed by HUSEMANN et al. 2014). FERREIRA et al. (2016) studied the damselfly Coenagrion puella throughout the Western Palaearctic. They found no pattern in Europe – including the Iberian Peninsula and Russian Caucasus, but found that North African populations were highly distinct regardless of the genetic markers used. EL MOKHEFI at al. (2016) also found that the pine processionary moth (Thaumetopoea pityocampa (Denis & Schiffermüller, 1775)) potentially has several North African refugia. The close connection we find between North Africa and the Iberian Peninsula is supported by several previous studies. JOGER et al. (2007: reptiles), HABEL et al. (2011: butterflies), and LEFEBVRE et al. (2016: termites) show that western North African and Iberian populations are often closely related, even if they are distinct today. Similarly, HUSE-MANN et al. (2014) considered the Tunisian-Algerian-Moroccan northern regions to have comprised a single Atlanto-Mediterranean glacial refugium together with the Iberian Peninsula.

Although the divergence time analysis in BEAST is uncertain, our results do indicate the timing of the events that have shaped the current distribution of the A. cyanea complex in Western Palearctic. SCHNEIDER et al. (2015) suggested that the original spilt between A. vercanica and A. cyanea occurred in the Hyrcanian Forest in what is today northern Iran. Our analysis dates this split to have occurred approximately 0.82 mya, which coincides with climatic changes at the beginning of the Cromerian 'complex' in the 'North Western Europe stage' (EHLERS & GIB-BARD 2008, GIBBARD & COHEN 2008, HOUMARK-NIELSEN et al. 2012), and the end of the Krinitisian/Krinitsa period in the 'Russian Plain Stages' (GIBBARD & COHEN 2008). During the interglacial periods in the mid-Cromerian (ca 0.7-0.6 mya: GIBBARD & COHEN 2008, HOUMARK-NIELSEN et al. 2012), A. cyanea may have spread west and southwest through Anatolia and the Middle East to North Africa, and further into the Maghreb region along the southern Mediterranean coast to Tunisia, Algeria and Morocco. During the various inter-glacial cycles in the mid-Cromerian (EHLERS & GIBBARD 2008, GIBBARD & COHEN 2008, HOUMARK-NIELSEN et al. 2012), populations in the Middle East and eastern North Africa may have become extinct leading to genetic isolation and eventually the split between A. cyanea Caucasus and the ancestor of A. cyanea North Africa + Europe around 0.6 mya perhaps further enforced by the onset of the Donian Glaciation in the 'Russian Plain Stages' (mid-Cromerian) ca 0.5–0.6 mya (GIBBARD & COHEN 2008). Prior to the Holsteinian interglacial period (ca 0.4-0.39 mya, (GIBBARD & COHEN 2008, HOUMARK-NIELSEN et al. 2012)) A. cyanea probably dispersed from western North Africa to the Iberian Peninsula, and the populations on either side of the Strait of Gibraltar probably formed a loosely connected meta-population until they were finally separated ca 0.41 mya at the start of the Holsteinian interglacial period (GIBBARD & COHEN 2008, HOUMARK-NIELSEN et al. 2012). Finally, A. cyanea probably spread from the Iberian Peninsula throughout Europe following the isolation in the Iberian Peninsula. However, our data does not provide sufficient resolution to allow us to assess whether this dispersal has happened during some warming periods in the Saale Glaciation (ca 390-130 kya), the Eemian Interglacial (ca 130–115 kya), the Weichsel Glaciation (ca 115–12 kya), or the warming in early Holocene (ca 11.7 kya) (Gibbard & Cohen 2008, Houmark-Nielsen et al. 2012, HELMENS 2014). The relatively high genetic variation within A. cyanea Europe indicates that the European population has not been through a recent genetic bottleneck like e.g. Sympetrum vulgatum (HINOJOSA et al. 2017) or Nehalennia speciosa (BERNARD et al. 2011). The colonization of Europe from a North African-Iberian refugium may fit a general picture (e.g. HUSEMANN et al. 2014), but the scenario is not completely uncontroversial. HUSEMANN et al. (2014) also mention North Africa – Italy (via Sicily) as a common colonization route, and DAPPOR-TO et al. (2011) demonstrate this pattern for the butterfly Maniola jurtina (Linneaus, 1758). Nevertheless, the Italian specimen in our data set clearly group with northern European taxa in a separate subgroup (with strong support in the Bayesian analyses) (Figs 2A-C, 3; Supplementary Figures S2-S3), despite the fact that the current North African populations are closer to Sicily than they are to the Iberian Peninsula (Fig. 1).

Despite our reasonably clear results, we must urge some caution as *A. cyanea* occurs beyond our sampled area throughout Eastern Europe and deep into Russia to the southern Ural Mountains (KALKMAN & KITANOVA 2015). It is quite possible that populations in Russia and Ukraine are more closely related to *A. cyanea* Caucasus than to *A. cyanea* Europe. However, we do have specimens from eastern Poland and the Swedish island Öland in the Baltic Sea, which clearly belong in *A. cyanea* Europe. Some previous studies on other taxa, that have shown an East-West split in Europe, have placed specimens from Öland (TINNERT et al. 2016) and eastern Poland (SZTENCEL-JABLONKA et al. 2015) in eastern groups clearly distinct from Western Europe.

5. Conclusions

The *Aeshna cyanea* complex appears to be an excellent model system for studying the phylogeography and responses to Quaternary climate and landscape changes of Western Palaearctic insects, and genome based studies with a broad taxon sampling of the group should be a priority.

6. Acknowledgements

We are grateful to the following people for support. Ole Fogh Nielsen, Mogens Holmen, Nicholas Bell and Erik Dylmer (all Denmark), and Tomasz Karasek (Poland) for providing specimens for DNA extractions. Annie Brandstrup (Aarhus University) assisted MD in the DNA lab. We thank Dominik Vondráček for reviewing and commenting on the manuscript. Funding was provided by The Research Fund under the Danish Ministry for Culture (Grant: FORM.2015-0023), the 15 June Foundation (Grant: 2015-A-89), and Schjøtz-Christensen's Memorial Fund (Denmark).

7. References

- ARTISS, T. 2004. Phylogeography of a facultatively migratory dragonfly, *Libellula quadrimaculata* (Odonata: Anisoptera). – Hydrobiologica **515**: 225–234. doi: 10.1023/B:HYDR.0000027332.5 7786.9d
- ASKEW, R.R. 2004. The Dragonflies of Europe (revised edition). Harley Books, Colchester, 308 pp. doi: 10.1111/j.1096-3642. 2006.00265.x
- BANDELT H.J., FORSTER P., ROHL A. 1999. Median-joining networks for inferring intraspecific phylogenies. – Molecular Biology and Evolution 16: 37–48. doi: 10.1093/oxfordjournals.molbev. a026036
- BERNARD R., HEISER M., HOCHKIRCH A., SCHMITT T. 2011. Genetic homogeneity of the Sedgling *Nehalennia speciosa* (Odonata: Coenagrionidae) indicates a single Würm glacial refugium and trans-Palaearctic postglacial expansion. Journal of Zoological Systematics and Evolutionary Research **49**: 292–297. doi: 10.1111/j.1439-0469.2011.00630.x
- BESOLD J., SCHMITT T. 2014. More northern than ever thought: refugia of the Woodland Ringlet butterfly *Erebia medusa* (Nymphalidae: Satyrinae) in Northern Central Europe. – Journal of Zoological Systematics and Evolutionary Research **53**: 67–75. doi: 10.1111/jzs.12076
- KALKMAN V.J., KITANOVA. D. 2015. Aeshna cyanea (Müller, 1764). Pp. 153–155 in: Boudot J.P., Kalkman, V.J. (eds) 2015. Atlas of European Dragonflies and Damselflies. KNNV Publishing, The Netherlands. 381 pp.
- BOUCKAERT R., VAUGHAN T.G., BARIDO-SOTTANI J., DUCHÊNE S., FOURMENT M., GAVRYUSHKINA A., HELED J., et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. – PLoS computational biology 15: e1006650. doi: 10.1371/journal.pcbi.1006650
- BROWER A.V.Z. 1994, Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* – inferred from patterns of mitochondrial-DNA evolution. – Proceedings of the National Academy of Sciences of the United States of America **91**: 6491–6499. doi: 10.1073/pnas.91.14.6491
- BYBEE S.M., CÓRDOBA-AGUILAR A., DURYEA M.C., FUTAHASHI R., HANSSON B., LORENZO-CARBALLA M.O., SCHILDER R., STOKS R., SUVOROV A., SVENSSON G.E., SWAEGERS J., TAKAHASHI Y., WATTS P.C., WELLENREUTHER M. 2016. Odonates as a bridge between ecology and evolutionary genomics. – Frontiers in Zoology 13: 46. doi: 10.1186/s12983-016-0176-7
- CHOBANOV D.P., KAYA S., GRZYWACZ B., WARCHALOWASKA-ŚLIWA E., ĆIPLAK B. 2016. The Anatolio-Balkan phylogeographic fault: a snapshot from the genus *Isophya* (Orthoptera, Tettigoniidae). – Zoologica Scripta **46**: 165–179. doi: 10.1111/zsc.12194
- DAPPORTO L., HABEL J.C., DENNIS R.L.H., SCHMITT T. 2001. The biogeography of the western Mediterranean: elucidating contradictory distribution patterns of differentiation in *Maniola jurtina* (Lepidoptera: Nymphalidae). – Biological Journal of the Linnean Society **103**: 571–577. doi: 10.1111/j.1095-8312.2011.01639.x
- DANECK H., FÉR T., MARHOLD K. 2015. Glacial survival in northern refugia? Phylogeography of the temperate shrub *Rosa pendulina*

L. (Rosaceae): AFLP vs. chloroplast DNA variation. – Biological Journal of the Linnean Society **119**: 704–718. doi: 10.1111/ bij.12619

- DE LATTIN G. 1964. Die Verbreitung der sibirischen Faunenelemente der Lepidopteren in der Westpaläarktis. – Natur und Museum Frankfurt am Main **94**: 505–514.
- DE LATTIN G. 1967. Grundriss der Zoogeographie. Verlag Gustav Fischer, Stuttgart, 602 pp.
- DUPUIS J.R., ROE A.D., SPERLING F.A.H. 2012. Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. – Molecular Ecology 21: 4422–4436. doi: 10.1111/j.1365-294X.2012.05642.x
- EL MOKHEFI M., KERDELHUÉ C., BURBAN C., BATTISTI A., CHAKALI G., SIMONATO M. 2016. Genetic differentiation of the pine processionary moth at the southern edge of its range: contrasting patterns between mitochondrial and nuclear markers. – Ecology and Evolution 6: 4274–4288. doi: 10.1002/ece3.2194
- EHLERS J., GIBBARD P. 2008. Extent and chronology of Quaternary glaciation. Episodes 33: 211–218. doi: 10.18814/epiiugs/2008/ v31i2/004
- EXCOFFIER L., LAVAL G., SCHNEIDER S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. – Evolutionary bioinformatics online 1: 47. doi: 10.1177/117693430500100003
- FÉLIX M. A., VIERSTRAETE A., VANFLETEREN J. 2001. Three biological species closely related to *Rhabditis (Oscheius) pseudodolichura* Körner in Osche, 1952. Journal of Nematology 33: 104–109.
- FERREIRA S., BOUDOT J.-P., EL HAISSOUFI M., ALVES P.C., THOMPSON D.J., BRITO J.C., WATTS P.C. 2016. Genetic distinctiveness of the damselfly *Coenagrion puella* in North Africa: an overlooked and endangered taxon. – Conservation Genetics 17: 985–991. doi: 10.1007/s10592-016-0826-5
- FREELAND J.R., CONRAD K.F. 2002. Genetic similarity within and among populations of the Variable and Azure damelflies (*Coenagrion pulchellum* and *C. puella*). – Hydrobiologica **479**: 69–73. doi: 10.1023/A:1021058231151
- GIBBARD P., COHEN K.M. 2008. Global chronostratigraphical correlation table for the last 2.7 million years. – Episodes 31: 243– 247. doi: 10.18814/epiiugs/2008/v31i2/011
- GRATTON P., KONOPIŃSKI M.K., SBORDONI V. 2008. Pleistocene evolutionary history of the Clouded Apollo (*Parnassius mnemosyne*): genetic signatures of climate cycles and a 'time-dependent' mitochondrial substitution rate. – Molecular Ecology **17**: 4248– 4262. doi: 10.1111/j.1365-294X.2008.03901.x
- HABEL J.C., HUSEMANN M., RÖDDER D., SCHMITT T. 2011. Biogeographical dynamics of the Spanish Marbled White *Melanargia ines* (Lepidoptera: Satyridae) in the Western Mediterranean: does the Atlanto-Mediterranean refuge exist? – Biological Journal of the Linnean Society **104**: 828–837. doi: 10.1111/j.1095-8312.2011.01760.x
- HAMMOUTI N., SCHMITT T., SEITZ A., KOSUCH J., VEITH M. 2010. Combining mitochondrial and nuclear evidences: a refined evolutionary history of *Erebia medusa* (Lepidoptera: Nymphalidae: Satyrinae) in Central Europe based on the COI gene. – Journal of Zoological Systematics and Evolutionary Research 48: 115– 125. doi: 10.1111/j.1439-0469.2009.00544.x
- HEBERT P.D.N, CYWINSKA A., BALL S.L., DEWAARD J.R. 2003. Biological identifications through DNA barcodes. – Proceedings of the Royal Society B 270: 313–321. doi: 10.1098/rspb.2002.2218
- HELMENS K.F. 2014. The Last Interglacial–Glacial cycle (MIS 5-2) re-examined based on long proxy records from central and northern Europe. Quaternery Sciences Review 86: 115–143. doi: 10.1016/j.quascirev.2013.12.012
- HEWITT G.M. 1999. Post-glacial re-colonization of European biota. – Biological Journal of the Linnean Society 68: 87–112. doi: 10.1111/j.1095-8312.1999.tb01160.x
- HEWITT G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. – Philosophical Transactions of the Royal Society of London Series B: Biological Sciences **359**: 183–195. doi: 10.1098/rstb.2003.1388

- HIJMANS R.J., GUARINO L., MATHUR P. 2012. DIVA-GIS Version 7.5 Manual (PDF, software and spatial data available at: http://www. diva-gis.org/download)
- HINOJOSA J.C., MARTIN R., MAYNOU X., VILA R. 2017. Molecular taxonomy of the *Sympetrum vulgatum* (Odonata: Libellulidae) complex in the West Palaearctic. – European Journal of Entomology **114**: 373–378. doi: 10.14411/eje.2017.048
- HOUMARK-NIELSEN M., KNUDSEN K.L., NOE-NYGÅRD N. 2012. Istider og Mellemistider. Pp. 255–303 in:W LARSEN G. (ED.). Naturen i Danmark. Geologien. (2nd edition). Gyldendal, Copenhagen.
- HUEMER P. 2011. Pseudo-endemism and cryptic diversity in Lepidoptera – case studies from the Alps and the Abruzzi. – Eco Mont-Journal on Protected Mountain Areas Research and Management **3**: 11–18. doi: 10.1553/eco.mont-3-1s11
- HUSEMANN M., SCHMITT T., ZACHOS F.E., ULRICH W., HABEL J.C. 2014. Palaearctic biogeography revisited: evidence for the existence of a North African refugium for Western Palaearctic biota. – Journal of Biogeography 41: 81–94. doi: 10.1111/ jbi.12180
- JOGER U., FRITZ U., GUICKING D., KALYABINA-HAUF S., NAGY Z.T., WINK M. 2007 Phylogeography of western Palaearctic reptiles – Spatial and temporal speciation patterns. – Zoologischer Anzeiger 246: 293–313. doi: 10.1016/j.jcz.2007.09.002
- JÖDICKE R., LANGHOFF P., MISOF B. 2003. The species-group taxa in the Holarctic genus *Cordulia*: a study in nomenclature and genetic differentiation (Odonata: Corduliidae). – International Journal of Odonatology 7: 37–52. doi: 10.1080/13887890.2004.9748193
- KAMP L., PASINELLI G., MILANESI P., DROVETSKI S.V., KOSIŃSKI Z., KOSSENKO S., ROBLES H., SCHWEIZER M. 2019. Significant Asia– Europe divergence in the middle spotted woodpecker (Aves, Picidae). – Zoologica Scripta 48: 17–32. doi: 10.1111/zsc.12320
- KAJTOCH L., CIEŚLAK E., VARGA Z., PAUL W., MAZUR M.A., SRAMKÓ G., KUBISZ D. 2016. Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation. – Biodiversity and Conservation 25: 2309–2339. doi: 10.1007/s10531-016-1065-2
- KOHLI M., SAHLÉN G., KUHN W.R., WARE J.L. 2018. Extremely low genetic diversity in a circumpolar dragonfly species, *Somatochlora sahlbergi* (Insecta: Odonata: Anisoptera). – Scientific Reports 8: 15114. doi: 10.1038/s41598-018-32365-7
- KODANDARAMAIAH U., SIMONSEN T.J, BROMILOW S., WAHLBERG N., SPERLING F.A.H. 2013. Deceptive single-locus taxonomy and phylogeography: *Wolbachia* mediated discordance between morphology, mitochondria and nuclear markers in a butterfly species. – Ecology and Evolution 3: 5167–5176. doi: 10.1002/ ece3.886
- KROSCH M.N., CRANSTON P.S. 2012. Non-destructive DNA extraction from Chironomidae, including of fragile pupal exuviae, extends analysable collections and enhances vouchering. CHIRONOMUS Newsletter on Chironomidae Research 25: 22–27.
- KUMAR S., STECHER G., TAMURA K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. – Molecular Biology and Evolution 33: 1870–1874. doi: 10.1093/ molbev/msw054
- LEFEBVRE T., VARGO E.L., ZIMMERMANN M., DUPONT S., KUTNIK M., BAGNÈRES A.-G. 2016. Subterranean termite phylogeography reveals multiple postglacial colonization events in southwestern Europe. – Ecology and Evolution 6: 5987–6004. doi: 10.1002/ ece3.2333
- LOUY D., HABEL J.C., ABADJIEV S., SCHMITT T. 2013. Genetic legacy from past panmixia: high genetic variability and low differentiation in disjunct populations of the Eastern Large Heath butterfly. – Biological Journal of the Linnean Society **110**: 281–290. doi: 10.1111/bij.12144
- Lowe C.D., HARVEY I.F., THOMPSON D.J., WATTS P.C. 2008. Strong genetic divergence indicates that congeneric damselflies *Coenagrion puella* and *C. pulchellum* (Odonata: Zygoptera: Coenagrionidae) do not hybridise. – Hydrobiologica **605**: 55–63. doi: 10.1007/s10750-008-9300-9

- MARZAHN E., MAYER W., JOGER U., ILGAZ Ć., JABLONSKI D., KINDLER C., KUMLUTAŞ Y., NISTRI A., SCHNEEWEISS N., VAMBERGER M., ŽAGAR A., FRITZ U. 2016. Phylogeography of the *Lacerta viridis* complex: mitochondrial and nuclear markers provide taxonomic insights. – Journal of Zoological Systematics and Evolutionary Research 54: 85–105. doi: 10.1111/jzs.12115
- MEIRMANS P.G., HEDRICK P.W. 2011. Assessing population structure: F_{st} and related measures. – Molecular Ecology Resources 11: 5–18. doi: 10.1111/j.1755-0998.2010.02927.x
- MUTANEN M., HAUSMANN A., HEBERT P.D.N., LANDRY J.-F., DE WAARD J.R., HUEMER, P. 2012. Allopatry as a Gordian Knot for Taxonomists: Patterns of DNA Barcode Divergence in Arctic-Alpine Lepidoptera. – Plos One 7: e47214. doi: 10.1371/journal. pone.0047214
- NEBEL C., GAMAUF A., HARING E., SEGELBACHER G., VILLERS A, ZA-CHOS F.E. 2015. Mitochondrial DNA analysis reveals Holarctic homogeneity and a distinct Mediterranean lineage in the Golden eagle (*Aquila chrysaetos*). – Biological Journal of the Linnean Society **116**: 328–340. doi: 10.1111/bij.12583
- NORMAND S., RICKLEFS R.E., SKOV F., BLADT J., TACKENBERG O., SVENNING J.-C. 2011. Postglacial migration supplements climate in determining plant species ranges in Europe. – Proceedings of the Royal Society B 278: 3644–3653. doi: 10.1098/rspb. 2010.2769
- PAUĆULOVA L., ŠEMELÁKOVÁ M., MUTANEN M., PRISTAS P., PANIGAJ L. 2016. Searching for the glacial refugia of *Erebia euryale* (Lepidoptera, Nymphalidae) – insights from mtDNA- and nDNAbased phylogeography in the Western Carpathians. – Journal of Zoological Systematics and Evolutionary Research 55: 118– 128. doi: 10.1111/jzs.12156
- QUINZIN M.C., NORMAND S., DELLICOUR S., SVENNING J.-C., MARDU-LYN P. 2017. Glacial survival of trophically linked boreal species in northern Europe. – Proceedings of the Royal Society B 284: 20162799. doi: 10.1098/rspb.2016.2799
- RONQUIST F., HUELSENBECK J., TESLENKO M. 2012. Draft MrBayes version 3.2 Manual: Tutorials and Model Summaries. Online publication available from the authors.
- SADEGHI S., KYNDT T., DUMONT H.J. 2010. Genetic diversity, population structure and taxonomy of *Calopteryx splendens* (Odonata: Calopterygidae). European Journal of Entomology 107: 137–146. doi: 10.14411/eje.2010.019
- SCHMITT T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. – Frontiers in Zoology 4: 11. doi: 10.1186/1742-9994-4-11
- SCHMITT T., RAKOSY L., ABADJIEV S., MUELLER P. 2007. Multiple differentiation centres of a non-Mediterranean butterfly species in south-eastern Europe. – Journal of Biogeography 34: 939–950. doi: 10.1111/j.1365-2699.2006.01684.x
- SCHNEIDER T., SCHNEIDER E., SCHNEIDER J., VIERSTRAETE A., DUMONT H.J. 2015. Aeshna vercanica sp. nov. from Iran with a new insight into the Aeshna cyanea group (Odonata: Aeshnidae). – Odonatologica 44: 81–106.
- SIMONSEN T.J., HUEMER P. 2014. Phylogeography of *Hepialus hu-muli* (L.) (Lepidoptera: Hepialidae) in Europe: short distance vs. large scale postglacial expansions from multiple Alpine refugia and taxonomic implications. Insect Systematics and Evolution 45: 209–249. doi: 10.1163/1876312X-44032104
- SIMONSEN T.J., BROWN R.L., SPERLING F.A.H. 2008. Tracing an invasion: Phylogeography of the invasive cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) in southeastern USA based on mitochondrial DNA. – Annals of the Entomological Society of America **101**: 899–905. doi: 10.1093/aesa/101.5.899
- SIMONSEN T.J., MOORE M.D., DUPONT S.T., STEVENS M. 2019. Testing DNA barcodes against morphology for the 'tripectinate *Abantiades*' (Lepidoptera: Hepialidae) reveals a complex relationship between COI sequence data and morphology. – Austral Entomology 58: 792–799. doi: 10.1111/aen.12395
- SLOVÁK M., KUĆERA J., TURIS P., ZOZOMOVÁ-LIHOVÁ J. 2012. Multiple glacial refugia and postglacial colonization routes inferred for a woodland geophyte, *Cyclamen purpurascens*: patterns concordant with the Pleistocene history of broadleaved and coniferous tree species. – Biological Journal of the Linnean Society **105**: 741–760. doi: 10.1111/j.1095-8312.2011.01826.x

- SZTENCEL-JABLONKA A., MAZGAJSKI T. D., BURY S., NAJBAR B., RYBACKI M., BOGDANOWICZ W., MAZGAJSKA J. 2015. Phylogeography of the smooth snake *Coronella austriaca* (Serpentes: Colubridae): evidence for a reduced gene pool and a genetic discontinuity in Central Europe. – Biological Journal of the Linnean Society **115**: 195–210. doi: 10.1111/bij.12496
- TINNERT J., HELLGREN O., LINDBERG J., KOCH-SCHMIDT P., FORSMAN A. 2016. Population genetic structure, differentiation, and diversity in *Tetrix subulata* pygmy grasshoppers: roles of population size and immigration. – Ecology and Evolution 6: 7831–7846. doi: 10.1002/ece3.2520
- URSENBACHER S., CARLSSON M., HELFER V., TEGELSTROM H., FUMA-GALLI, L. 2006. Phylogeography and Pleistocene refugia of the adder (*Vipera berus*) as inferred from mitochondrial DNA sequence data. – Molecular Ecology **15**: 3425–3439. doi: 10.1111/j.1365-294X.2006.03031.x
- VILA M., VIDAL-ROMANI J.R., BJÖRKLUND M. 2005. The importance of time scale and multiple refugia: Incipient speciation and admixture of lineages in the butterfly *Erebia triaria* (Nymphalidae). – Molecular Phylogenetics and Evolution **36**: 249–260. doi: 10.1016/j.ympev.2005.02.019
- VON CRÄUTLEIN M., LEINONEN P.H., KORPELAINEN H., HELANDER M., VÄRE H., SAIKKONEN K. 2019. Postglacial colonization history reflects in the genetic structure of natural populations of *Festuca rubra* in Europe. – Ecology and Evolution **9**: 3661–3674. doi: 10.1002/ece3.4997
- WEEKERS P.H.H., DE JONCKHEERE J., DUMONT H. 2001. Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographical patterns in representatives of the genus *Calopteryx* (Insecta: Odonata) of the West Mediterranean and adjacent West European zone. – Molecular Phylogenetics and Evolution 20: 89–99. doi: 10.1006/mpev.2001.0947
- WINDMAISSER T., KATTARI S., HEUBL G., REISCH C. 2016. Glacial refugia and postglacial expansion of the alpine-prealpine plant species *Polygala chamaebuxux*. – Ecology and Evolution 6: 7809–7819. doi: 10.1002/ece3.2515

Authors' contributions

T.J.S. and K.O. designed the study, secured funding and collected material. M.D. carried out laboratory work, data mining and the initial analyses. T.J.S. carried out the bulk of the analyses and drafted the text. All authors contributed to the Discussion and the final version of the paper.

Electronic Supplement Files

at http://www.senckenberg.de/arthropod-systematics

ASP_78-2_Simonsen_et_al_Electronic_Supplements.zip DOI: 10.26049/ASP78-2-2020-06/1

File 1: Supplementary Data S1.nex – MrBayes Nexus file of the combined COI and ITS region data set)

File 2: Supplementary Figures.pdf — Fig S2: Tree from the 100 million generation analysis in MrBayes. — Fig. S3: Tree from the unconstrained 100 million generation analysis in BEAST.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Arthropod Systematics and Phylogeny

Jahr/Year: 2020

Band/Volume: 78

Autor(en)/Author(s): Simonsen Thomas J., Olsen Kent, Djernaes Marie

Artikel/Article: <u>The African-Iberian connection in Odonata: mtDNA and ncDNA based</u> phylogeography of Aeshna cyanea (Müller, 1764) (Odonata: Aeshnidae) in Western Palaearctic 309-320